

Claims

RECEIVED BY
ART 34/ANDT

1. Method for producing a protein comprising the steps:
 - (a) providing a nucleic acid sequence coding for the protein in which a heterologous nucleic acid sequence is inserted on the 3' side of the translation start codon in the correct reading frame, said heterologous nucleic acid sequence being selected such that a stem-loop structure is formed on the 3' side of the translation start codon at a distance of 6-30 nucleotides,
 - (b) providing an expression system suitable for expressing the protein and
 - (c) introducing the nucleic acid sequence according to (a) into the expression system according to (b) under such conditions that the protein is synthesized.
2. Method as claimed in claim 1 additionally comprising the isolation of the protein.
3. Method as claimed in claim 1 or 2,
characterized in that,
the inserted heterologous nucleic acid sequence has a length of up to 201 nucleotides.
4. Method as claimed in claim 3,
characterized in that
the inserted heterologous nucleic acid sequence has a length of up to 45 nucleotides.

5. Method as claimed in one of the claims 1 to 4,
characterized in that
the stem-loop structure is formed at a distance of 12-21 nucleotides on the 3' side of the start codon.
6. Method as claimed in one of the claims 1 to 5,
characterized in that
the length of the stem in the stem-loop structure is in the range of 4-12 nucleotides.
7. Method as claimed in one of the claims 1 to 6,
characterized in that
the region of the heterologous nucleic acid sequence that is on the 5' side of the stem-loop structure does not itself form a secondary structure and cannot form a secondary structure with the 5' untranslated region of the nucleic acid sequence coding for the protein to be produced.
8. Method as claimed in one of the claims 1 to 7,
characterized in that
the region of the heterologous nucleic acid sequence that is on the 5' side of the stem-loop structure and on the 3' side of the ATG start codon has a GC content of < 50 %.
9. Method as claimed in one of the claims 1 to 8,
characterized in that
an *in vitro* expression system is used.

10. Method as claimed in claim 9,
characterized in that
a prokaryotic *in vitro* expression system is used.
11. Method as claimed in claim 10,
characterized in that
the prokaryotic *in vitro* expression system comprises lysates of gram-negative bacteria in particular of *Escherichia coli* or of gram-positive bacteria in particular *Bacillus subtilis*.
12. Method as claimed in claim 9,
characterized in that
a eukaryotic *in vitro* expression system is used.
13. Method as claimed in claim 12,
characterized in that
the eukaryotic *in vitro* expression system comprises lysates of mammalian cells in particular of rabbits, reticulocytes, human tumour cell lines, hamster cell lines, or other vertebrate cells, in particular oocytes and eggs of fish and amphibia as well as insect cell lines, yeast cells, algal cells or extracts of plant seedlings.
14. Method as claimed in one of the claims 1 to 8,
characterized in that
a prokaryotic *in vivo* expression system is used.
15. Method as claimed in claim 14,
characterized in that
a prokaryotic host cell is used as the expression system.

16. Method as claimed in claim 15,
characterized in that
a gram-negative prokaryotic host cell, in particular an *E. coli* cell or a gram-positive prokaryotic host cell, in particular a *Bacillus subtilis* cell is used.
17. Method as claimed in claim 14,
characterized in that
a eukaryotic host cell is used as the expression system.
18. Method as claimed in claim 17,
characterized in that
a yeast cell, an insect cell or a vertebrate cell in particular an amphibian, fish, bird or mammalian cell is used.
19. Method as claimed in claim 14,
characterized in that
a non-human eukaryotic host organism is used as the expression system.
20. Method as claimed in one of the claims 1 to 19,
characterized in that
the nucleic acid sequence coding for the protein is provided by cloning, recombination or/and amplification.
21. Method as claimed in claim 20,
characterized in that
the provision comprises a two-step PCR.

22. Method as claimed in one of the claims 1 to 21,
characterized in that
the nucleic acid sequence coding for the protein to be produced or/and the
heterologous nucleic acid sequence at least partially have a codon usage
adapted to the respective expression system.
23. Method as claimed in one of the claims 1 to 22,
characterized in that
the heterologous nucleic acid sequence contains a section coding for a
purification domain or/and a section coding for a proteinase recognition
domain.
24. Reagent for producing a protein comprising
(a) a nucleic acid sequence that is heterologous to the nucleic acid sequence
coding for the protein which can be inserted into the protein-coding
nucleic acid sequence in the correct reading frame and which can form a
stem-loop structure at a distance of 6-30 nucleotides on the 3' side of the
translation start codon, and
(b) an expression system that is suitable for producing the protein.